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18 SUBJECT TERMS (Continue on reverse if necessary and identify by block number):
Biomembranes; Biomolecular; Membrane Proteins; Infrared Spectroscopy; Raman Spectroscopy; Nanometer Lithography

19 ABSTRACT (Continue on reverse if necessary and identify by block number)

Abstract: Fourier transform infrared spectroscopy and Raman spectroscopy are being used to study the structure and stability of biomembranes from Archaebacteria as well as to probe their function at the single residue level. In the case of bacteriorhodopsin, structural stability can be probed by examining the effect of single amino acid substitutions. A method of utilizing these crystalline membranes as 2-dimensional masks for nanometer molecular lithography has been developed. Methods for incorporating active membrane components into ordered arrays fabricated on conducting films and addressing these components is an additional objective of this project. (f.12)

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ANNUAL REPORT-YEAR 1

CONTRACT: N00014-88-K-0464 **R&T CODE 441-D018**

PRINCIPAL INVESTIGATOR: Kenneth J. Rothschild

CONTRACTOR: Boston University

CONTRACT TITLE: *Biophysical Study of Archaebacteria Biomembrane and Possible Application in Biomaterials*

START DATE: July 1, 1988

RESEARCH OBJECTIVES: The objective of this project is to study the structure and molecular basis for structural stability in Archaebacterial membranes. An additional objective is to study the utilization of these membranes in the production of new materials which are fabricated on the nanometer scale. We are focussing on the 2-dimensionally crystalline protein from the S-layer of *Sulfolobus acidocaldarius* which is resistant to low pH and high temperature. An additional system is bacteriorhodopsin from *Halobacterium halobium*, a light driven proton pump, which also exhibits structural stability and two-dimensional ordering.

PROGRESS (Year 1): In the past 10 months we have made progress in several areas as detailed below:

(1) Utilisation of Archaebacterial Membranes for Molecular Device Fabrication. We have extended our method of nanometer lithography using the S-layer from *Sulfolobus Acidocaldarius* (1-5). This method enables us to produce a 2-dimensional array of nanometer size holes in a thin metal layer (1,2). We have now demonstrated (3,4) that by controlling the charge on the exposed carbon and metal surfaces we can produce selective binding of ferritin, an electron dense protein. The selective binding of biomolecules into a 2-d array provides us with a new tool for nanometer device fabrication. For example, ferritin can be conjugated with a variety of molecules including antibodies, thus enabling additional specificity to be obtained.

(2) FTIR Study of the Structure of S-layer Protein. We have begun studies on the structure of the S-layer from *Sulfolobus acidocaldarius*. Our initial measurements indicate that well oriented films of S-layer can be formed. Infrared absorption in the amide I and II region indicates an unusual β-type secondary structure. Studies are currently being carried out to probe the orientation and temperature dependence of this structure. The recent demonstration that absorption

from single groups in the spectrum of biomembranes can be detected using deconvolution will enable these studies be made at the level of single residues (8).

(3) Scanning Tunneling Microscopy of Biomembranes. Two Nanoscope II scanning tunneling microscope systems (Digital Instruments, Inc.) have been setup for this project in Dr. K. Douglas and N.A. Clark's laboratory at the University of Colorado. These systems will be used as a probe of both the membrane protein array structure as well as a potential means of addressing hybrid biomolecular structures. Initial measurements on fabricated nanostructures has begun (4). A key problem is the development of a metal overcoating which enables tunneling currents to be detected and is also suitable for nanometer lithography

(4) Studies on the Properties of Site-directed Mutants of Bacteriorhodopsin. In collaboration with Dr. H.G. Khorana's laboratory at MIT we are studying the effects of site-directed mutations on bacteriorhodopsin (5-7). For this purpose, we have assembled a nanosecond UV/visible flash spectroscopy system capable of characterizing the photocycle of bacteriorhodopsin mutants. These measurements, being carried out by Dr. Dunach in our laboratory, will enable us to probe the structural stability of these mutants as well as to identify unusual photocycle behavior.

INVENTIONS (Year 1): A patent which was filed prior to the start date was received (see publication 1) on a method for parallel fabrication of nanometer scale multi-device structures.

WORK PLAN (Year 2):

1. Improve methods of isolation and purification of S-layer from *Sulfolobus Acidocaldarius* in order to eliminate impurities that interfere with obtaining uniform nanometer arrays in thin metal films. Scale-up production using an improved environmental chamber.

2. Determine the structure and overall orientation of the S-layer protein using polarized FTIR spectroscopy and Raman spectroscopy. Determine structural changes as a function of temperature using FTIR spectroscopy.

3. Continue development of technique to bind proteins selectively into nanometer arrays.

4. Develop methods to image and address biological molecules incorporated in nanometer arrays.

5. Continue to characterize the properties of site-specific mutants of bacteriorhodopsin.

PUBLICATIONS (Year 1):

1. U.S. Patent # 4,802,951 Clark, N.A., Douglas, K., and Rothschild, K.J. "Method for Parallel Fabrication of Nanometer Scale Multi-Device Structures" Feb 7, 1989.

2. Douglas, K., Clark, N.A. and Rothschild, K.J., "Nanometer Molecular Lithography" Molecular Electronic Devices (eds. F.L. Carter, R.E. Siatokowski and H. Woltjen) Elsevier Science Publishers B.V. (North-Holland) (1988)

3. Douglas, K., Clark, N.A. and Rothschild, K.J. "Biomolecular/Solid-state Nanoheterostructures" Applied Physics Letters (To be submitted)

4. Douglas, K., Clark, N.A. and Rothschild, K.J., "Composite Biomolecular/Solid-State Nanostructures" Submitted to Materials Research Society, 1989 Fall Meeting

5. Douglas, K., Clark, N.A. and Rothschild, K.J. "Biomolecular/Solid State Nanoheterostructures" Bulletin of the American Physical Society **34**, 633 (1989)

6. Braiman, M.S., Mogi, T., Marti, Stern, L.J., Khorana, H.G., & Rothschild, K.J. *Biochemistry* **27**, 8516-8520 (1988).

7. Rothschild, K.J., Braiman, M.S., Mogi, T., Stern, L.J. and Khorana, H.G. *FEBS Letts.* (in Press) (1989)

8. Rothschild, K.J., Braiman, M.S., Bousche, O., He, Y-W. and Degrip, W.J. *Proc. Soc. Opt. Eng.* **1057**, 44-48 (1989)

TRAINING ACTIVITIES: There is currently one undergraduate, one Senior Research Associate and one technician involved in this project. The demographic data regarding the project personnel are:

Women or minorities-2

Non-citizens-1

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